

Appl. No. 09/703,809
Amrdr. Dated January 15, 2004
Reply to Office Action of July 25, 2003

Amendments to the Specification:

Please replace the paragraph beginning at page 3, line 22 to page 4, line 6, with the following rewritten paragraph:

--Testis has important endocrine (hormonal) functions and is the site for the production of haploid spermatozoa from undifferentiated stem cells, a process called spermatogenesis. Mutations in some specialized ~~transcriptional~~ transcription activator proteins, such as A-myb and CREM, cause male infertility and show defects in spermatogenesis. The identification of tissue-specific human general transcription factor would bridge an important gap between the generality for general transcription factor function and the specificity of gene-specific ~~transcriptional~~ transcription activator protein function. If such factors were testis-specific, they would be expected to regulate patterns of gene expression that are important in the endocrine, spermatogenic and other functions of this organ. The present invention satisfies a need in the art for new compositions for polynucleotide sequences and encoded polypeptide products, immunological reagents and other derived materials in terms of providing unique reagents for the detection of defects in testis function such as idiopathic male infertility or other syndromes, for detection of dysfunctional patterns of gene expression and as reagents that can modulate gene expression. --

Please replace the paragraphs beginning at page 5 line 20 to page 7, line 6, with the following rewritten paragraphs:

-- The present invention, in a general and overall sense, concerns the isolation and characterization of a novel ~~transcriptional~~ transcription factor gene, ALF and carboxy terminal variable region. One embodiment of the present invention is a purified nucleic acid segment that encodes a protein having an amino acid sequence as shown in Figure 2, in accordance with SEQ ID NO.: 2. Another embodiment of the present invention is a purified nucleic acid segment, further defined as including a nucleotide sequence in accordance with SEQ ID NO.: 1.

The present invention also concerns the isolation and characterization of a novel ~~transcriptional~~ transcription factor gene, SALF and a carboxy terminal variable region. One embodiment of the present invention is a purified nucleic acid segment that encodes a protein

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having an amino acid sequence as shown in Figure 3, in accordance with SEQ ID NO.:4. Another embodiment of the present invention is a purified nucleic acid segment, further defined as including a nucleotide sequence in accordance with SEQ ID NO.: 3. The 3' variable region that ALF and SALF have in common is encoded by the nucleic acid segment in accordance with SEQ ID NO.: 5 and expressed as an amino acid sequence as shown in SEQ ID NO.: 6.

In one embodiment the purified nucleic acid segment includes the nucleotide sequence of SEQ ID NOS.: 1, 3 and 5. As used herein, the term "nucleic acid segment" and "DNA segment" are used interchangeably and refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a "purified" DNA or nucleic acid segment as used herein, refers to a DNA segment that includes novel ~~transcriptional~~ transcription factor genes, ALF, SALF and a carboxy terminal variable coding sequence, yet is isolated away from, or purified free from, total genomic DNA, for example, total cDNA or human genomic DNA. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments and recombinant vectors, including, for example, plasmids, cosmids, phage, viruses and the like.

Similarly, a DNA segment encoding an isolated or purified novel ~~transcriptional~~ transcription factor genes, ALF, SALF and a carboxy terminal variable coding sequence, gene refers to a DNA segment including ALF, SALF and a carboxy terminal variable coding sequence isolated substantially away from other naturally occurring genes or protein encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein, polypeptide or peptide encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences, cDNA sequences or combinations thereof. "Isolated substantially away from other coding sequences" means that the gene of interest, in this case ALF, SALF and a carboxy terminal variable coding sequence, forms the significant part of the coding region of the DNA segment. Of course, this refers to the DNA segment as originally isolated and does not exclude genes or coding regions later added by the hand of man to the segment.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode novel ~~transcriptional~~ transcription factor genes, ALF, SALF and a carboxy terminal variable coding sequence genes, and that include within the amino acid sequence an amino acid sequence in accordance with SEQ

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ID NO.: 2. Moreover, in other particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode a gene which includes within its amino acid sequence the amino acid sequence of a ALF, SALF and a carboxy terminal variable coding sequence. --

Please replace the paragraph beginning at page 11 line 4 to line 19, with the following rewritten paragraph:

--Another embodiment of the present invention is a purified composition comprising a polypeptide having an amino acid sequence in accordance with SEQ ID NOS.: 2, 4 or 2 or 4 with 6. The term "purified" as used herein, refers to a ~~transcriptional~~ transcription factor protein composition, wherein the ALF, SALF or ALF and SALF having the variable region proteins are purified to any degree relative to its naturally-obtainable state, i.e., in this case, relative to its purity within a eukaryotic cell extract, or a testis sample. A cell for the isolation of ALF, SALF or variants thereof is a cell of testicular origin, however, these proteins may also be isolated from patient specimens, recombinant cells, tissues, isolated subpopulations of tissues, and the like, as will be known to those of skill in the art, in light of the present disclosure. Purified ALF, SALF or variants thereof also refer to polypeptides having the amino acid sequence of SEQ ID NOS.: 2, 4, 2 and 6 or 4 and 6, free from the environment in which it may naturally occur. One may also prepare fusion proteins and peptides, e.g., where the ALF, SALF or variable portion coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (e.g., proteins that may be purified by affinity chromatography and enzyme label coding regions, respectively).--

Please replace the paragraphs beginning at page 11 line 4 to line 19, with the following rewritten paragraphs:

--FIG. 1 depicts the cDNA sequence of ALF (SEQ ID NO.: 1);

FIG. 2 depicts the corresponding deduced amino acid sequence of ALF (SEQ ID NO.: 2), standard one-letter abbreviations for amino acids is used;

FIG 3 depicts the cDNA sequence of SALF (SEQ ID NO.: 3);

FIG 4 depicts the corresponding deduced amino acid sequence of ALF (SEQ ID NO.: 4), standard one-letter abbreviations for amino acids is used;

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FIG 5 depicts the cDNA sequence of an alternative 3'-coding and untranslated region for both ALF and SALF (SEQ ID NO.: 5);

FIG 6 depicts the corresponding deduced amino acid sequence of ALF (SEQ ID NO.: 6), standard one-letter abbreviations for amino acids is used; --

Please replace the paragraphs beginning at page 16, line 12 to page 17, line 9, with the following rewritten paragraphs:

--The term "ALF" (TFIIA α / β -like factor) refers to the nucleotides essentially as set forth (SEQ ID NO. 1) or amino acid sequences essentially as set forth (SEQ ID NO.: 2). The term "SALF" (Stoned B/TFIIA α / β -like factor) refers to the nucleotides essentially as set forth (SEQ ID NO.: 3) or amino acid sequence essentially as set forth (SEQ ID NO.: 4). The term "alternative carboxy terminal domain" refers to nucleotide essentially as set forth (SEQ ID NO.: 5) and amino acid sequences essentially as set forth (SEQ ID NO.: 6) It is to be understood that alternative carboxy terminal sequence is present as an alternative 3'-coding and untranslated region that can be found on some ALF or SALF transcripts or cDNAs and is meant to be included or implied in all references to the term "ALF and SALF", without referring to this alternative sequence explicitly each time.

The terms "a sequence essentially as set forth in SEQ ID NO.: (#)", "a sequence similar to", "nucleotide sequence" and similar terms, with respect to nucleotides, refers to sequences that substantially correspond to any portion of the sequences in SEQ ID NOS.: 1, 3 and 5. These terms refer to synthetic as well as naturally-derived molecules and includes sequences that possess biologically, immunologically, experimentally, or otherwise functionally equivalent activity, for instance with respect to hybridization by nucleic acid segments, or the ability to encode all or portions of ALF or SALF activities. Naturally, these terms are meant to include information in such a sequence as specified by its linear order.

The terms "a sequence essentially as set forth in SEQ ID NO.: (#)", "a sequence similar to", "amino acid sequence" and similar terms, with respect to amino acids, refers to peptides, polypeptides, proteins, fragments, fusions, derivatives and alterations thereof that substantially correspond to any portion of the sequences in SEQ ID NOS.: 2, 4 and 6. These terms refer to synthetic as well as naturally-derived molecules and includes sequences that possess

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biologically, immunologically, experimentally, or otherwise functionally equivalent activities, for instance, segments of amino acids which possess immunological activity as an antigenic determinant. Naturally, these terms are meant to include information in such a sequence as specified by its linear order. --

Please replace the paragraph beginning at page 23, line 16 to line 29, with the following rewritten paragraph:

--One aspect of the present invention is the polynucleotide sequences essentially as set forth as SEQ ID NOS.: 1, 3 and 5, and in FIGS. 1 and 3, the complement of these sequences, the RNA versions of both DNA strands and the information otherwise contained within the linear sequence of these polynucleotide sequences and fragments thereof. In the case of nucleic acid segments, sequences for use with the present invention are those that have greater than about 50 to 60% homology with any portion of the polynucleotide sequences described herein, sequences that have between about 61% and about 70%; sequences that have between about 71 and about 80%; or between about 81% and about 90%; or between 91% and about 99%; or which contain nucleotides that are identical, functionally equivalent, or functionally irrelevant, with respect to the nucleotides present in SEQ ID NOS.: 1, 3 and 5 are considered to be essentially similar. Also encompassed within the present invention are nucleic acids that encode polypeptides that are at least 40% identical or similar to the amino acid sequences shown in SEQ ID NOS.: 2, 4 and 6, and in FIGS.: 2, 4 and 6. --

Please replace the paragraph beginning at page 24, line 9 to line 14, with the following rewritten paragraph:

--Included within the invention are DNA or RNA segments including oligonucleotides, polynucleotides and fragments thereof, including DNA or RNA or nucleic acid-like sequences of genomic or synthetic origin, single or double stranded. The invention includes nucleic acid molecules, or nucleic acid-like molecules that are able to hybridize to the sequences in SEQ ID NOS.: 1, 3 and 5, under stringent or under permissive hybridization conditions, or to the complement of said sequences.--

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Please replace the paragraph beginning at page 27, line 8 to line 23, with the following rewritten paragraph:

--One aspect of the invention is the protein, polypeptide, oligopeptide, or amino acid sequences or fragments thereof, of ALF and SALF, essentially as set forth in SEQ ID NOS. 2, 4 and 6. Sequences that have greater than about 40-50% homology with any portion of the amino acid sequences described herein, sequences that have between about 51% and about 60%; sequences that have between about 61% and about 70% sequences that have between about 70 and about 80%; or between about 81% and about 90%; or between 91% and about 99%; or those that contain amino acids that are identical, functionally equivalent, or functionally irrelevant, for instance those specified by conservative, evolutionarily conserved, and degenerate substitutions, with respect to the amino acid sequences presented in SEQ ID NOS. 2, 4 and 6 are included. The invention thus applies to ALF and SALF sequences, or fragments thereof, and nucleic acids which encode such polypeptides, such as those of other species. Reference is particularly, but not exclusively, made to the conserved N- (amino acids 1-54) and C-terminal (amino acids 417-478) regions of ALF and SALF, in contrast to similarity throughout the entire length. The invention thus encompasses amino acid sequences, or amino acid-like molecules, that are sufficient in any regard to mimic, substitute for, or interfere with the ALF or SALF amino acid sequences, or fragments thereof. --

Please replace the paragraph beginning at page 34, line 6 to line 19, with the following rewritten paragraph:

--Other detection methods could include those based on direct hybridization, such as include fluorescent in situ hybridization (FISH), in situ hybridization, DNA "chip", or "microarray" hybridization technology, Southern and Northern hybridization analysis, RNA dot blot hybridization, dipstick, pin, dot blot, in situ PCR, and other techniques. Others methods may be based on annealing between short, typically 15-30 nucleotide, complementary DNA or RNAs followed by enzymatic extension, such as PCR analysis of genomic DNA or cDNA, reverse-transcriptase mediated PCR using RNA. Such oligonucleotides are derived from the sequences in SEQ ID NOS. 1, 3, or 5, or their complements, or from flanking 5'- or 3' cDNA or genomic regions, or from introns or alternatively spliced exons, alleles, promoter or enhancer

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regions, and so forth. Visualization of the results from such methods is accomplished by a number of methods, including light or fluorescent microscopy, autoradiographic detection, or detection based on ethidium bromide stained agarose gels, DNA sequencing, and so forth. These and other techniques would be those available to and recognized by those skilled in the art. --

Please replace the paragraphs beginning at page 44, line 6 to page 45, line 7, with the following rewritten paragraphs:

--The 5'-end of SALF was amplified by PCR (40 cycles) using 4 μ l of the human placental cDNA library (Clontech) with primer 2a2-6 (5'-AGTAACCCGAATGCTTAA-3') (SEQ ID NO.: 8) and a commercially available library-specific adapter primer AP1 (Clontech). The resulting products were reamplified (35 cycles) with primer 2a2-8 (5'-ATGCTAGCTGAACCACTG-3') (SEQ ID NO.: 9) and a commercially available nested library-specific adaptor primer AP2 (Clontech) used to obtain a 2,930 bp product, which was subcloned into the pCRII cloning vector (Invitrogen) to form pRACE4 (FIG 7A). Sequence analysis of this and EST ID 256637 constitute SEQ ID NO.: 3. Human SALF cDNAs were identified by PCR amplification (35 cycles) of 1.1 and 0.9 kb products from human placenta, liver and testis "Marathon" cDNA libraries (Clontech) using 25 pmol of the upstream primer 2a2-1 (5'-AGAAATTCCTCTGATTG-3') (SEQ ID NO.: 7) and the downstream primers 2a2-6 and 2a2-8. The 1.1 and 0.9 kb products derived from the liver cDNA library were subcloned into pGEM-T Easy (Promega). Sequence analysis of the liver-derived products shows that they are identical to those present in both SEQ ID NO.: 3. These products are diagrammed in FIG 7A, and shown in FIG 7C.

ALF sequences were isolated by PCR (35 cycles) using 4 μ l of the human testis cDNA library (Clontech) with the gene-specific primer 2a2-20 (5'-CCAGAAGGTAGAATTGCGGGTTGCTGTAGC-3') (SEQ ID NO.: 12) and primer AP1 (Clontech), and reamplified with 2a2-22 (5'-GGAGTTTGAAGTGCCCAGGTCTGCTGTGG-3') (SEQ ID NO.: 19) and primer AP2 (Clontech). The 369 bp amplification product is subcloned into pGEM-T Easy (Promega) to form pRACE22. The resulting clone (pRACE22; FIG 7B) contains 298 bp that are identical to SALF and a 35 bp 5'-end that is unique to SEQ ID NO.: 1. A full-length ALF PCR product is amplified (35 cycles) from 4 ml of the testis cDNA library

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(Clontech) using primer 2a2-17 (5'- GGTGCTGTCATGGCCTGCCTCAACCCGG-3') (SEQ ID NO.: 13), located within the unique 5'-end of ALF, and primer AP1 (Clontech). The resulting 1.7 bp fragment is subcloned into pGEM-T Easy to form pRACE17 (FIG 7B). The sequence of the resulting clone is identical to the composite SALF sequence except for its unique 5'-end and a longer poly(A) tail (~90 nucleotides) which begins four nucleotides downstream of the poly(A) tail in SALF. The composite sequences of pRACE17 and pRACE22 are SEQ ID NO.: 1. --